(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 January 2003 (30.01.2003)

PCT

(10) International Publication Number
WO 03/008591 A1

(51) International Patent Classification[7]: C12N 15/54

(21) International Application Number: PCT/KR02/01344

(22) International Filing Date: 16 July 2002 (16.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2001/42931 16 July 2001 (16.07.2001) KR

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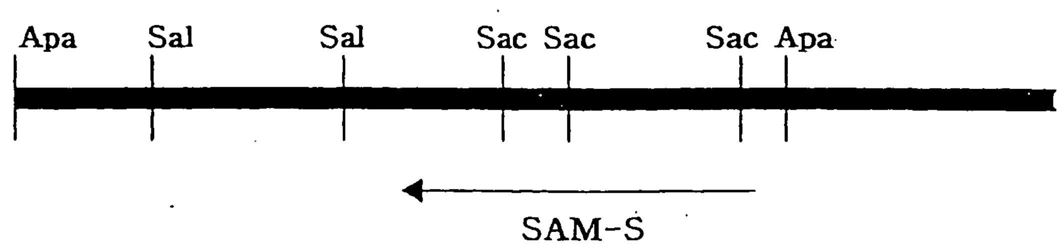
(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ADENOSYLMETHIONINE SYNTHETASE FROM STREPTOMYCES SP., GENE SEQUENCES CODING THE SAME AND METHOD FOR MASS PRODUCTION OF SECONDARY METABOLITES INCLUDING ANTIBIOTICS THEREOF



(57) Abstract: Disclosed is an isolated nucleotide sequence encoding an enzyme catalyzing biosynthesis of SAM (SAM-s) and its amino acid sequence. Also, the present invention provides a method for mass production of a useful secondary metabolite including antibiotics using the isolated nucleotide sequence and SAM, where SAM acts as a methyl group donor.

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ADENOSYLMETHIONINE SYNTHETASE FROM STREPTOMYCES SP., GENE SEQUENCES CODING THE SAME AND METHOD FOR MASS PRODUCTION OF SECONDARY METABOLITES INCLUDING ANTIBIOTICS THEREOF

TECHNICAL FIELD

The present invention relates to S-adenosyl-L-methionine synthetase and a nucleotide sequence encoding the same, and more particularly, to an isolated nucleotide sequence encoding an enzyme catalyzing biosynthesis of SAM (SAM-s) from adenosyl triphosphate (ATP) and methionine, and its amino acid sequence. Also, the present invention is concerned with a method for mass production of a useful secondary metabolite including antibiotics using the nucleotide sequence and SAM.

PRIOR ART

S-adenosyl-L-methionine (SAM) is well known to play a critical role in cell growth and differentiation, essential for survival of living organisms including human beings. In living cells, SAM acts as a methyl group donor as well as a precursor for an amninopropyl group in a biosynthesis pathway of polyamine, where the methyl group and the polyamine are utilized in primary and secondary metabolisms.

It has been reported that SAM positively or negatively affects growth of bacteria including *E. coli* and *Bacillus subtilis*, thus causing their

life cycles to change in a manner of inhibiting cell growth or stimulating morphological differentiation.

In addition, the biological function of SAM is also found to be essential for primary and secondary metabolisms in plants and animals. Especially, it has been reported that SAM as a methyl group donor affects differentiation, causing morphological changes in plant or animal cells.

On the other hand, spectinomycin, which is an antibotic derived from *Streptomyces spectabilis*, belongs to an aminoglycoside family and is composed of one sugar and two methyl groups originated from a methyl group donor, SAM.

DISCLOSURE OF THE INVENTION

Based on the fact that methyl groups of spectinomycin are derived from SAM, inventors of the present invention conducted intensive and thorough research into effects of SAM on biosynthesis of spectinomycin, resulting in the finding that SAM positively affects the biosynthesis of antibiotics, thereby increasing their production yield.

Therefore, it is an object of the present invention to provide an isolated nucleotide sequence encoding an enzyme catalyzing biosynthesis of SAM from *Streptomyces spectabilis* ATCC 27741 and an amino acid sequence translated from the isolated nucleotide sequence.

It is another object of the present invention to provide a method of increasing production of a useful secondary metabolite including antibiotics using SAM.

In accordance with the present invention, the first object is achieved by isolating a gene encoding an enzyme catalyzing SAM biosynthesis,

which is derived from *S. spectabilis*, by obtaining a PCR product of 4.0 kb from a gene library of *S. spectabilis* using PCR, and confirming presence of a gene of about 1.2 kb in the PCR product encoding an enzyme catalyzing SAM biosynthesis, by sequencing the PCR product assaying activity of its translational product.

In accordance with the present invention, the second object is achieved by producing SAM, which is synthesized by the translational product of the isolated nucleotide sequence or that which is commercially available, having an ability to stimulate production of an antibiotic.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a restriction map of a gene encoding an enzyme catalyzing SAM biosynthesis (SAM-s) carried in a recombinant plasmid, pJWK0012, which is originated from an *E. coli* plasmid;

Fig. 2 is a result of a homology search comparing the amino acid sequence of "SAM-s" of the present invention to SAM synthetases from other microorganisms, obtained from GeneBank database, USA;

Fig. 3 is a graph showing an ability of "SAM-s" to synthesize SAM, using HPLC, where commercially available SAM is used as a control;

Fig. 4 is a graph showing an effect of SAM produced by "SAM-s" on production yield of actinorhodin in *S. lividans* TK23 transformed with

a gene encoding "SAM-s";

Fig. 5 is photograph showing an effect of SAM produced by "SAM-s" on production yield of actinorhodin in *S. lividans* TK23 treated with commercially available SAM; and

Fig. 6 is a graph showing an effect of SAM on production yield of undecylprodigiosin in *S. lividans* TK23.

BEST MODES FOR CARRYING OUT THE INVENTION

In accordance with the present invention, there is provided an enzyme catalyzing biosynthesis of S-adenosyl-L-methionine having an amino acid sequence consisting of the sequence shown in SEQ ID NO. 2, which is derived from *Streptomyces spectabilis* ATCC 27741.

In accordance with the present invention, there is provided an isolated nucleotide sequence consisting of the sequence shown in SEQ ID NO. 1, which encodes the amino acid sequence of SEQ ID NO. 2.

In accordance with the present invention, there is also provided a method of producing a useful secondary metabolite including antibiotics in a *Streptomyces* species-originated transformant into which a gene encoding an enzyme catalyzing biosynthesis of SAM is introduced, thereby improving productivity of the useful secondary metabolite.

In accordance with the present invention, there is further provided a method of producing a useful secondary metabolite including antibiotics by directly adding SAM to culture medium containing antibiotic-producing bacteria, thereby improving productivity of the useful secondary metabolite.

In the present invention, preferable examples of the useful secondary metabolite include antibiotics, such as polyketide antibiotics, anti-cancer agents, and vermicides.

In the present invention, a gene encoding an enzyme catalyzing SAM biosynthesis (SAM-s) is isolated from cosmid clones containing genes encoding enzymes stimulating biosynthesis of spectinomycin, which is obtained from a cDNA library of *S. spectabilis* ATCC 27741, where a 3.9 kb clone is primarily obtained. The nucleotide sequence of the 3.9 kb clone is partially determined by performing nucleotide sequencing, and its homology to known SAM synthetases, which mediate biosynthesis of SAM using ATP and methionine as substrates, is examined, based on the obtained nucleotide sequence, indicating the possible presence of a gene encoding an enzyme catalyzing SAM biosynthesis. The 3.9 kb clone is demonstrated to carry the gene encoding the enzyme catalyzing SAM biosynthesis by in vitro assaying the activity of its translated product.

In accordance with the present invention, a portion of the 3.9 kb clone, containing the gene encoding an enzyme catalyzing SAM biosynthesis (SAM-s), is introduced into *Streptomyces* species to assay production yield of an antibiotic, actinorhodin, and also, the effect of SAM on production of antibiotics is further investigated through direct treatment of cells with SAM, thereby demonstrating that SAM is effective in improving productivity of secondary metabolites including antibiotics.

In embodiments of the present invention, *Streptomyces lividans* TK23, which is commercially available, is transformed with the gene encoding "SAM-s", and the resulting transformant exhibits mass production of actinorhodin, demonstrating that SAM is effective in

enhancing antibiotic productivity of cells. Herein, application of SAM for high production of antibiotics is not limited to the transformant and the antibiotic, actinorhodin, but the effectiveness of SAM on production of antibiotics can be achieved with all *Streptomyces* species transformed with a gene encoding an enzyme catalyzing SAM biosynthesis.

In addition, when SAM is directly added to culture medium containing antibiotic-producing bacteria, productivity of antibiotics is increased 5 to 10 times, and especially, production of polyketide antibiotics is significantly increased.

The present invention will be explained in more detail with reference to the following examples in conjunction with the accompanying drawings. However, the following examples are provided only to illustrate the present invention, and the present invention is not limited to them.

EXAMPLE 1 : Cloning of a gene encoding an enzyme capable of SAM biosynthesis from cosmid clones of *Streptomyces spectabilis* ATCC 27741

Genes encoding enzymes catalyzing biosynthesis of an antibiotic are typically located together in a specific region of a genome. Therefore, there was used two cosmid clones harboring 30 to 40 kb fragment, which carries a gene family consisting of genes encoding enzymes participating in spectinomycin biosynthesis and may also include a gene encoding methyltransferase enzyme, one of enzymes mediating spectinomycin biosynthesis, which functions to transfer methyl groups. After digestion of the two cosmid clones with restriction enzymes,

Southern Blotting was performed using metK gene, having high homology to methlytransferase at the nucleotide sequence level, as a probe.

As a result of Southern Blotting, a positive spot was observed, indicating a 3.9 kb fragment inserted into a BamHI site of pHCG121. 3.9 kb fragment was then subcloned into a BamHI site of pBluescript KS(+), giving a recombinant plasmid pHCG1647. From the subcloned 3.9 kb fragment, a 2.5 kb fragment, which is believed to carry a gene encoding an enzyme catalyzing SAM biosynthesis, was subcloned again into pBluescript KS(+) to form a recombinant plasmid pJWK0012.

EXAMPLE 2 : Determination of nucleotide sequence of the cloned 2.5 kb fragment and its corresponding amino acid sequence

In order to determine a nucleotide sequence of the cloned 2.5 kb fragment and its corresponding amino acid sequence, the 2.5 kb insert carried in pJWK0012 prepared in the Example 1 was digested with restriction enzymes, ApaI, SalI and SacI, and then subcloned, followed by nucleotide sequencing. Fig. 1 shows a restriction map of the 2.5 kb fragment in pJWK0012 and its translational orientation.

Based on the nucleotide sequence of the 2.5 kb fragment, its amino acid sequence was obtained through search using a Codon Preference program (Bibb, M. J. et al., Gene, 1984). As a result, the 2.5 kb fragment was found to have an open reading frame consisting of a coding region ranging from nt 835 to nt 2051, which may express a protein consisting of 464 amino acid. The translational product of the open reading frame was, in the present invention, called "SAM-s".

To investigate the homology of "SAM-s" to other known proteins, the amino acid sequence of "SAM-s" was compared to those of SAM synthetases of *Streptomyces coelicolor*, *Bacillus subtilis* and *Escherichia coli*, which were obtained from GeneBank DataBase (USA). With reference to Fig. 2, it was found that "SAM-s" shares high homology with other synthetases. Also, "SAM-s" of the present invention was found to have homology to some methyltransferases from microorganisms.

EXAMPLE 3 : Assay for activity of "SAM-s"

In order to analyze activity of "SAM-s", the gene encoding "SAM-s" was expressed in *E. coli*, and the resulting translational product, "SAM-s", was then isolated.

To express the gene encoding "SAM-s" in *E.coli*, the gene was inserted into a pET-21a vector, and then introduced into *E.coli* BL21. The expressed gene product, "SAM-s" was isolated using a His-Tag purification system. Thereafter, 10 to 50 μl of the enzyme solution containing the protein "SAM-s" was added to a reaction mixture containing 100 mM of Tris-HCl, 200 mM of KCl, 10 mM of $MgCl_2$, 1 mM DTT, 5 mM ATP, and 5 mM methionine, followed by incubation for 120 min at 30 °C. After the incubation, reaction products were analyzed through HPLC using Reverse C18 column. In this regard, the column loaded with sample was initially equilibrated with a solution of 0.1 M of NaH_2PO_4/acetonitrile at a ratio of 98:2 (V/V), pH 2.65. Then, a second solution comprising 0.15 M NaH_2PO_4/acetonitrile at a ratio of 74:26 (V/V) was applied with continuous mixing with the first solution, forming a concentration gradient.

As shown in Fig. 3, the product of the catalytic activity of the protein expressed in *E. coli* is proven to be SAM. That is, when the expressed protein is supplied with ATP and methionine as substrates, the product has an HPLC retention time identical to commercially available SAM, indicating that the protein expressed in *E. coli* has an activity to synthesize SAM using ATP and methionine as substrates.

EXAMPLE 4 : Effect of in vivo-synthesized SAM on productivity of actinorhodin in *S. lividans* TK23

The gene encoding "SAM-s" was first inserted into pWHM3, which is a shuttle vector between *E. coli* and *Streptomyces* species, giving an expression vector pSAM-s. The plasmid pSAM-s was then introduced into *S. lividans* TK23. The resulting transformant, *Streptomyces lividans* TK-23 harboring pSAM-s, was deposited in the Korean Culture Center of Microorganisms with accession No. KCCM 10397 on July 2, 2002. The transformant was incubated in one liter of a medium including 50 g of glycerol, 5 g of glutamic acid, 21 g of morpholinopropane sulfonic acid, 200 mg of $MgSO_4 7H_2O$, 100 mg of $CaCl_2 2H_2O$, 100 mg NaCl, 82 mg KH_2PO_4, 9 mg $FeSO_4 7H_2O$, and 2 ml of trace element solution, adjusted to pH 6.5. During incubation for 7 days at 28 °C, production yield of actinorhodin, which is a main antibiotic produced from *S. lividans* TK23, was analyzed. The results are shown in Fig. 4.

As apparent in Fig. 4, when SAM was over-produced in *S. lividans* TK23 through over-expression of "SAM-s", it was observed that production of actinorhodin in the transformant was enhanced to over six times in comparison with that of a wild type *S. lividans*.

EXAMPLE 5 : Effect of externally added SAM on productivity of actinorhodin

Based on the finding that in vivo over-expressed SAM positively affects production yield of actinorhodin in *S. lividans* TK23, an effect of SAM on productivity of actinorhodin was investigated when commercially available SAM is added directly to culture medium containing *S. lividans* TK23. As such, wild type *S. lividans* TK23 was treated with 1 mM of commercially available SAM.

The result is shown in Fig. 5, where actinorhodin produced in *S. lividans* TK23 treated with SAM, and the control not treated with SAM, indicated by a blue color. As shown in Fig. 5, it was found that *S. lividans* TK23 treated with SAM produces more actinorhodin than *S. lividans* TK23 not treated with SAM, demonstrating that SAM positively affects productivity of actinorhodin.

EXAMPLE 6 : Effect of SAM on productivity of undecylprodigiosin in *S. lividans* TK23

S. lividans TK23 transformed with the vector pSAM-s was incubated under the same culture conditions as those used for production of actinorhodin. To determine the amount of undecylprodigiosin produced, after adjusting pH to 12, absorbance was measured at 468 nm, and concentration of the antibiotic was calculated according to the following formula: concentration = OD value × 9.4673.

As apparent Fig. 6, productivity of undecylprodigiosin was verry high in comparison with a control not transformed with the vector pSAM-

s, indicating that SAM positively affects production yield of undecylprodigiosin.

EXAMPLES 7 to 13: Effect of SAM on productivity of antibiotics in *Streptomyces* species

TABLE 1
Culture medium and culture condition

Exp.	Antibiotic	*Streptomyces* sp.	Culture medium (/L) and culture condition
7	Avermectin	*S. avermitilis*	15g glucose, 0.5g asparagine, 0.5g K_2HPO_4, pH 7.0, 25 °C, incubation for 5 days
8	Monensin	*S. cinnamonensis*	2.5% glucose, 1.5% soybean meal, 0.3% $CaCO_3$, 0.03% $FeSO_47H_2O$, 0.003% $MnCl_24H_2O$, pH 7.0, 30 °C, incubation for 5 days
9	Spectinomycin	*S. spectabilis*	10g Maltose, 5g tryptone, 1g K_2HPO_4, 2g NaCl, pH7.0, 30 °C, incubation for 5 days
10	Doxorubicin	*S. peucetius*	60g Glucose, 8g yeast extract, 20g malt extract, 2g NaCl, 15g MOPS sodium salt, 0.1g $MgSO_4$, 0.01g $FeSO_47H_2O$, 0.01g $ZnSO_47H_2O$, pH 7.0, 30 °C, incubation for 5 days
11	Streptomycin	*S. griseus*	1 % glucose, 0.4 % peptone, 0.2 % meat extract, 0.2 % yeast extract, 0.5 % NaCl, 0.025 % $MgSO_47H_2O$, pH 7, 30 °C, incubation for 5 days
12	Tetracyclin	*S. aureofaciens*	3 % corn flour, 4 % corn steep liquor, 5 % corn starch, 0.7 % $(NH_4)_2SO_4$, 0.1 % NH_4Cl, 5 ppm $CoCl_2$, 0.9 % $CoSO_3$, 2 % rice bran oil, pH7, 28 °C, incubation

			for 5 days
13	Chlortetracyclin	*S. aureofaciens*	1 % sucrose, 1 % corn steep liquor, 0.2 % $(NH_4)_2HPO_4$, 0.2 % KH_2PO_4, 0.1 % $CaCO_3$, 0.025 % $MgSO_47H_2O$, 0.005 % $ZnSO_47H_2O$, 0.00033 % $CuSO_45H_2O$, 0.00033 % $MnCl_24H_2O$, incubation for 5 days

Each *Streptomyces* species was incubated in its corresponding culture medium according to Table 1, and treated with 1 mM of SAM (Sigma, USA). After incubation for 5 days, antibiotic concentration was measured in each culture medium. The results are given in Table 2, below. It was found that each *Streptomyces* species, as a treatment group, produces a much higher amount of its specific antibiotic than a control group not treated with SAM.

TABLE 2

Production amount of an antibiotic in *Streptomyces* sp. treated or not treated with SAM

Exp.	Antibiotic	*Streptomyces* sp.	Production amount of a control group (μg/ml)	Production amount of a treatment group (μg/ml)
7	Avermectin	*S. avermitilis*	5	25
8	Monensin	*S. cinnamonensis*	30	180
9	Spectinomycin	*S. spectabilis*	5	35
10	Doxorubicin	*S. peucetius*	38	300
11	Streptomycin	*S. griseus*	101	602
12	Tetracycline	*S. aureofaciens*	30	188

13	Chlortetracycline	*S. aureofaciens*	25	130

As shown in Table 2, *Streptomyces* species treated with SAM produced 5 to 10 times more antibiotic than the control, indicating that SAM positively affects production yield of various antibiotics.

INDUSTRIAL APPLICABILITY

As described hereinbefore, the present invention provides an isolated nucleotide sequence of a gene encoding an enzyme catalyzing biosynthesis of SAM, which is derived from *Streptomyces spectabilis* ATCC 27741, and its amino acid sequence. SAM, which is produced by the enzyme of the present invention or purchased commercially, is very effective in increasing productivity of various antibiotics. Therefore, the isolated nucleotide sequence of the present invention is capable of being utilized in mass production of secondary metabolites including antibiotics, and thus is very useful in pharmaceutical industries.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Suh, Joo-Won
San 38-2 Namdong, Yongin,
Kyunggido, 449-728, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR :	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Streptomyces lividans TK-23 harboring pSAM-s	KCCM-10397

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Jul. 2. 2002. (date of the original deposit)[1]

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :
Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Date: Jul. 9. 2002

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of i nternational depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

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CLAIMS

1. S-adenosyl-L-methionine synthetase, having an amino acid sequence consisting of the sequence shown in SEQ ID NO. 2, which is derived from *Streptomyces spectabilis* ATCC 27741.

2. A nucleotide sequence of SEQ ID NO. 1, encoding the amino acid sequence of claim 1.

3. A method of producing a useful secondary metabolite including antibiotics using a transformant into which a gene encoding S-adenosyl-L-methionine synthetase is introduced, thereby improving productivity of said useful secondary metabolite.

4. A method of producing a useful secondary metabolite including antibiotics comprising adding S-adenosyl-L-methionine to culture medium containing antibiotic-producing bacteria, thereby improving productivity of said useful secondary metabolite.

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FIG. 1

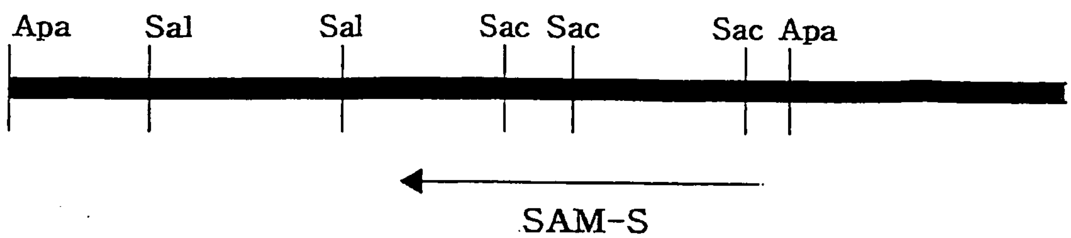

FIG. 2

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              1                                                  50
Mycobacterium MSEKGRLFTS ESVTEGHPDK ICDAISDSVL DALLAADPRS RVAVETLVTT GQVHVVGEVT TSAKEAFADI TNTVRARILE IGYDSSDKGF DGATCGVNIG
 Streptomyces ..MSRRLFTS ESVTEGHPDK IADQISDTIL DALLREDPTS RVAVETLITT GLVHVAGEVT T...KAYAPI AQLVREKILE IGYDSSKKGF DGASCGVSVS
  Escherichia ..MAKHLFTS ESVSEGHPDK IADQISDAVL DAILEQDPKA RVACETYVKT GNVLVGGEIT T...SAWVDI EEITRNTVRE IGYVHSDMGF DANSCAVLSA
     Bacillus MSKNRRLFTS ESVTEGHPDK ICDQISDSIL DEILKKDPNA RVACETSVTT GLVLVSGEIT T...STYVDI PKTVRQTIKE IGYTRAKYGF DAETCAVLTS
Staphylococcus MLNNKRLFTS ESVTEGHPDK IADQVSDAIL DAILKDDPNA RVACETTVT  GMALIAGEIS T...TTYVDI PKVVRETIKE IGYTRAKYGY DYETMAILTT
    Consensus .....rlfts esvtEGHPDK.!cDq!SD.!L DalL..DP.s RVA.ET.!tT Glvl!.GE!t t....a..di ...vR..!.E IGY..sd.G% da.sca!..a

              101                                                150
Mycobacterium IGAQSPDIAQ GVDTAHEARV EG.AADPLDS QGAGDQGLMF GYAINATPEL MPLPIALAHR LSRRLTEVRK NGVLPYLRPD GKTQVTIAYE DN.VPVRLDT
 Streptomyces IGAQSPDIAQ GVDTAYESRV EG.DEDELDR QGAGDQGLMF GYACDETPEL MPLPIHLAHR LSRRLSEVRK NGTIPYLRPD GKTQVTIEYD GD.KAVRLDT
  Escherichia IGKQSPDINQ GVDRADPLEQ .......... .GAGDQGLMF GYATNETDVL MPAPITYAHR LVQRQAEVRK NGTLPWLRPD AKSQVTFQYD DG.KIVGIDA
     Bacillus IDEQSADIAM GVDQALEARE GTMSDEEIEA IGAGDQGLMF GYACNETKEL MPLPISLAHK LARRLSEVRK EDILPYLRPD GKTQVTVEYD ENNKPVRIDA
Staphylococcus IDEQSPDIAQ GVDKALEYRD KD.SEEEIEA TGAGDQGLMF GYATNETETY MPLAIYLSHQ LAKRLSDVRK DGTLNYLRPD GKVQVTVEYD ENDNPVRID
    Consensus Ig.QSpDIaq GVd.A.e.r. .....deld. .GAGDQGLMF GYAc#eTpel MPlpI.laHr l..Rls#VRK ng.lpyLRPD gKtQVti.Y# d...pVriDt

              201                                                250
Mycobacterium VVISTQHAA. ..DIDLEKTL DPDIREKVLN TVLDDLAHET LDAST..VRVLVNPTGKFVL GGPMGDAGLT GRKIIVDTYG GWARHGGGAF SGKDPSKVDR
 Streptomyces VVVSSQHAS. ..DIDLESLL APDIREFVVE PELKALVEDG IKLETEGYRLLVNPTGRFEI GGPMGDAGLT GRKIIIDTYG GMSRHGGGAF SGKDPSKVDR
  Escherichia VVLSTQHSE. ..EIDQKS.L QEAVMEEIIK PILP...AEW LTSAT...KFFINPTGRFVI GGPMGDCGLT GRKIIVDTYG GMARHGGGAF SGKDPSKVDR
     Bacillus IVISTQHHP. ..EITLEQ.I QRNIKEHVIN PVVP...EEL IDEET...KYFINPTGRFVI GGPQGDAGLT GRKIIVDTYG GYARHGGGAF SGKDATKVDR
Staphylococcus IVVSTQHAE. ..DVTLEQ.I QEDIKAHVIY PTVP...ENL INEQT...K YINPTGRFVI GGPQGDAGLT GRKIIVDTYG GIARHGGGCF SGKDPTKVDF
    Consensus !viStQH.e. ..#!dle..l q..!.e.v.. pvl.....#. ....T...rf.!NPTGrFvl GGPmGDaGLT GRKII!DTYG G.aRHGGGaF SGKDpsKVDR

              301                                                350
Mycobacterium SAAYAMRWVA KNVVAAGLAE RVEVQVAYAI GKAAPVGLFV ETFGTETEDPVKIEKAIGEV FDLRPGAIIR DLNLLRPIYAPTA AYGHFGRTDV
 Streptomyces SAAYAMRWVA KNVVAAGLAS RCEVQVAYAI GKAEPVGLFV ETFGTNTIDTDKIEQAISEV FDLRPAAIIR SLDLLRPIYSQTA AYGHFGRSLP
  Escherichia SAAYAARYVA KNIVAAGLAD RCEIQVSYAI GVAEPTSIMV ETFGTEKVPSEQLTLLVREF FDLRPYGLIQ MLDLLHPIYKETA AYGHFGREH.
     Bacillus SAAYAARYVA KNIVAAELAD SCEVQLAYAI GVAQPVSISI NTFGSGKASEEKLIEVVRNN FDLRPAGIIK MLDLRRPIYKQTA AYGHFGRHDV
Staphylococcus SAAYAARYVA KNIVAAGLAD QCEVQLAYAI GVAEPVSIAI DTFGTGKVS GQLVEAVRKH FDLRPAGIIK MLDLKQPIYKQTA AYGHFGRTDE
 Synechocystis SAAYAARYVA KNIVAAGLAD KCEVQVSYAI GVARPVSVLI DTFGTGKVDEekl...!.e. F#LRPagiI. .L#Ll..piY.tA AYGHFGR.d.
    Consensus SAAYAaRyVA KN!VAAgLAd rcE!QvaYAI GvA.Pvs..! #TFGt.kvd.
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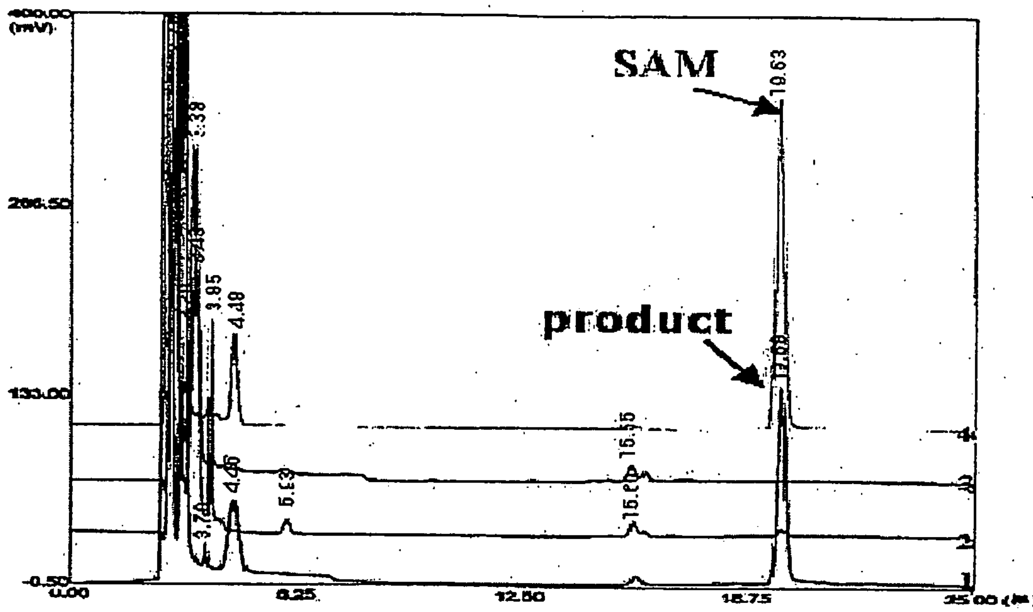
FIG. 3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(mV)
266.50
133.00
-0.50
0.00
6.25
12.50
18.75
25.00
SAM
19.63
product
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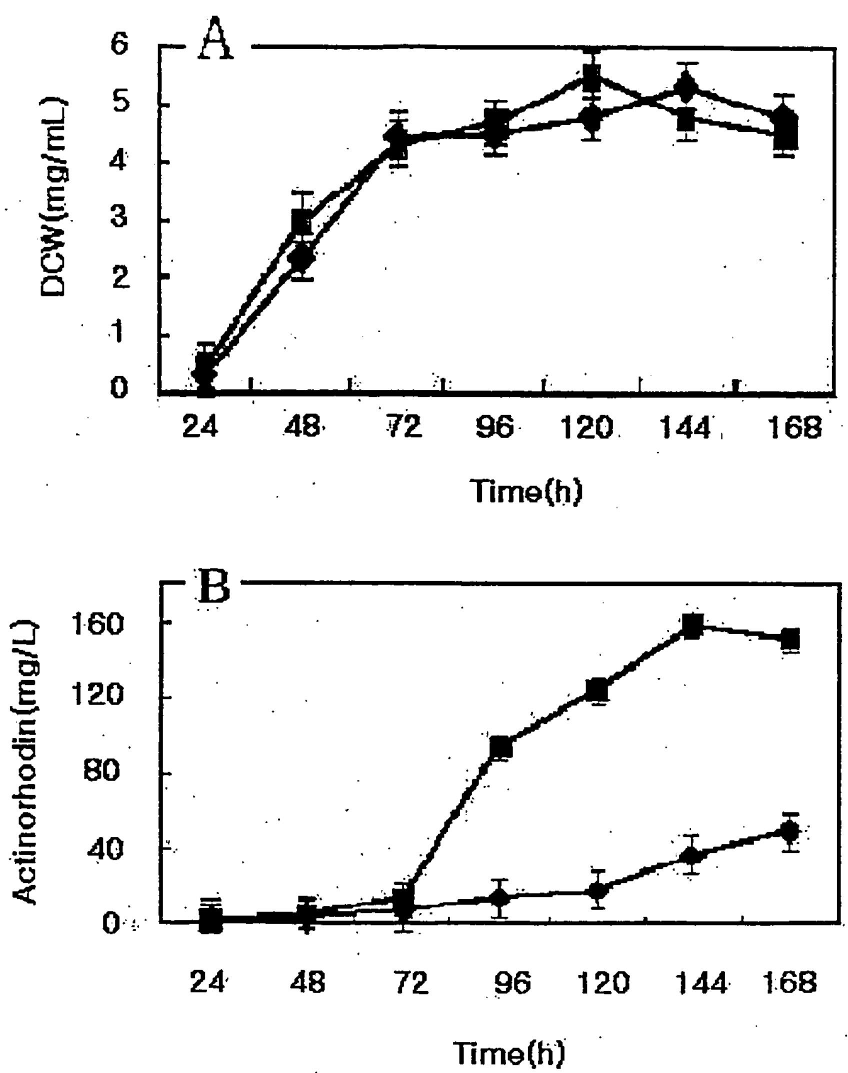
4.48
4.46
3.78
5.53
16.55
1
2
3
4

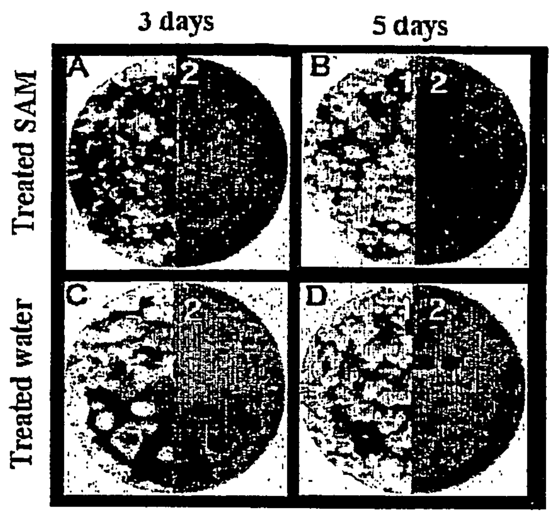
FIG. 4



[Note]

A : Cell growth
B : Produotion of antibiotios
■ : Transformant
◆ : Wild type

FIG. 5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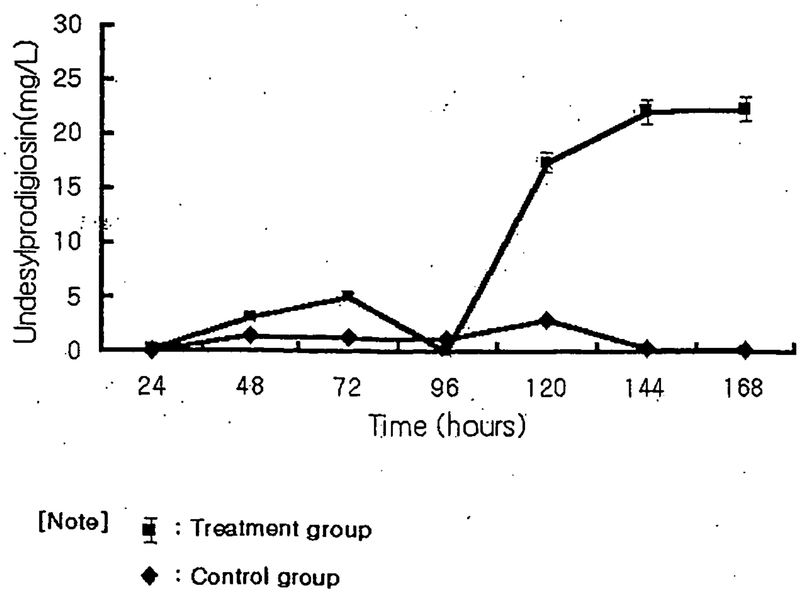
5 days
Treated SAM
Treated water
A
2
B
1
2
C
2
D
1
2

FIG. 6


30
25
20
15
10
5
0
Undesylprodigiosin(mg/L)
24
48
72
96
120
144
168
Time (hours)
[Note]
: Treatment group
: Control group

SEQUENCE LISTING

<110> SUH, Joo-Won

<120> Adenosylmethionine synthetase from Streptomyces sp. and gene sequences coding the same

<130> YL02007PCT

<140> PCT/KR02/01344
<141> 2002-07-16

<150> KR 2001-42931
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ccgacgcggt ggtgatggcc gccgccgtgg ccgacttccg cccggccgcg tacgccgccg     180

gaaagatcaa gaagcaggac ggcgaggacc cggccccgct caccctcgtc cgcaatccgg     240

atatcctcgc cgagctctcg ggcgaccgcc cgcgccccgg ccaggtcgtc gtcggcttcg     300

cggccgagac ggacaacgtg ctggccaacg gcgggccaag ctcgcccgca aggctgcgac     360

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ctccaggctc tctaaaccca ccctaaaccc gccaaatgcg ggccttgagc cctctggtgg     660

agaccgatcg tcgtatcgca caatgcagtg ccgcaggtca caggcctctc ttctgacgag     720

agaggtggcc atgcggccgt gtgtgaccga taaactggtc tcggacgtcg tcgagcgcag     780

ctctcggccc gtccatcaat gatcagccag cagccgctgc aaccacaggg agcgtg atg     839
                                                              Met
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Lys Ile Ala Asp Gln Ile Ser Asp Thr Ile Leu Asp Ala Leu Leu Arg
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gag gac ccg acg tcc cgg gtc gcc gtc gaa acg ctc atc acc acc ggc 983
Glu Asp Pro Thr Ser Arg Val Ala Val Glu Thr Leu Ile Thr Thr Gly
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Lys Lys Gly Phe Asp Gly Ala Ser Cys Gly Val Ser Val Ser Ile Gly
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Gly Leu Thr Gly Arg Lys Ile Ile Ile Asp Thr Tyr Gly Gly Met Ser
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Arg His Gly Gly Gly Ala Phe Ser Gly Lys Asp Pro Ser Lys Val Asp
290 295 300 305

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Arg Ser Ala Ala Tyr Ala Met Arg Trp Val Ala Lys Asn Val Val Ala
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325 330 335

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atc gac acg gac aag atc gag cag gcc atc agc gag gtc ttc gac ctc 1943
Ile Asp Thr Asp Lys Ile Glu Gln Ala Ile Ser Glu Val Phe Asp Leu
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cgc ccg gcc gcg atc atc cgc agc ctc gac ctg ctc cgc ccg atc tac 1991
Arg Pro Ala Ala Ile Ile Arg Ser Leu Asp Leu Leu Arg Pro Ile Tyr
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Gly Leu Val His Val Ala Gly Glu Val Thr Thr Lys Ala Tyr Ala Pro
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Ile Ala Gln Leu Val Arg Glu Lys Ile Leu Glu Ile Gly Tyr Asp Ser
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Thr Gln Val Thr Ile Glu Tyr Asp Gly Asp Lys Ala Val Arg Leu Asp
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Ala Leu Val Glu Asp Gly Ile Lys Leu Glu Thr Glu Gly Tyr Arg Leu
245 250 255

Leu Val Asn Pro Thr Gly Arg Phe Glu Ile Gly Gly Pro Met Gly Asp
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Ala Gly Leu Thr Gly Arg Lys Ile Ile Ile Asp Thr Tyr Gly Gly Met
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Asp Arg Ser Ala Ala Tyr Ala Met Arg Trp Val Ala Lys Asn Val Val
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Gly Lys Ala Glu Pro Val Gly Leu Phe Val Glu Thr Phe Gly Thr Asn
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Thr Ile Asp Thr Asp Lys Ile Glu Gln Ala Ile Ser Glu Val Phe Asp
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370 375 380

Tyr Ser Gln Thr Ala Ala Tyr Gly His Phe Gly Arg Ser Leu Pro Glu
385 390 395 400

Phe Thr Trp Glu Lys Thr Asp Arg Val Asp Ala Leu Arg Lys Ala Ala
405 410 415

Gly Leu Glu Ser
420

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/01344

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 15/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C12N 15/54, C12N 9/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the interntnational search (name of data base and, where practicable, search terms used)

CA, PubMed, Blast, Delphion, "S-adenosyl-L-methionine synthetase", "Streptomyces", "S-adenosyl-L-methionine", "antibiotic"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NCBI Accession # AF117274, 31 Mar. 1999.	1, 2
X	JP 09-224690 A2 (Shiseido Co., Ltd. & Takeda Chem. Ind., Ltd.), 02 Sep. 1997. See abstract.	3, 4
Y	Seno, E.T. et al., Antimicrob. Agents Chemother., 21(5), 758-63, 1982. See abstract.	3, 4
Y	Merali, S. et al., Proc. Natl. Acad. Sci. USA, 96(5), 2402-7, 1999. See abstract.	4

☐ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

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"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 NOVEMBER 2002 (25.11.2002)	25 NOVEMBER 2002 (25.11.2002)

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	LEE, Cheo Young Telephone No. 82-42-481-5594

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR02/01344

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 09-224690 A2	02 Sep. 1997	none	